Informative Title: Foodborne Outbreaks of Microbial Infection from Fresh Produce in Europe And North America: A Systematic Review of Data from This Millennium

Running Title: Foodborne Outbreaks from Fresh Produce

S.O. Aiyedun[1]*, B. A. Onarinde[1], M. Swainson[1] and R. A. Dixon[2].

[1]National Centre for Food Manufacturing, University of Lincoln Holbeach, PE12 7PT.

[2]School of Life Sciences, University of Lincoln, Lincoln, LN6 7TS.

*Corresponding author: Susanna Aiyedun; Address: National Centre for Food Manufacturing, University of Lincoln, Minerva House, Park Road, Holbeach, PE12 7PT United Kingdom; Tel.: +44 1406 493000; E-mail address: saiyedun@lincoln.ac.uk; susanaiyedun@gmail.com

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MISS SUSANNA OLUWASEYI AIYEDUN (Orcid ID : 0000-0001-6829-8075)

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Abstract

This study focuses on foodborne outbreaks of microbial infection associated with fresh produce in Europe and North America from this millennium. A total of 277 outbreaks with 44,524 individual cases were identified. Foodborne pathogens associated with the most outbreak frequency include *Cryptosporidium* (20.5%) in Europe and *Salmonella* (52.2%) in North America although *Norovirus* (54.3%) and *Salmonella* (61.3%) were associated with the number of cases in Europe and North America respectively. Vegetables were the most implicated fresh produce category with outbreak frequencies of 34.1% in Europe and 47.4% in North America. Increased consumption of fresh produce in Europe and North America, as measures to improve diets correlates with the increased fresh produce related outbreaks of microbial infection. This systematic review suggests the need for more rapid methodologies for traceback investigations in order to determine trends and epicentres of foodborne infections related to fresh produce.

INTRODUCTION

Globally, fresh produce form an integral part of a healthy human diet. A variety of fruits, vegetables, herbs, seeds and nuts belong to this category which can be consumed raw or in a minimally processed form (Machado-Moreira *et al*., 2019). Fresh produce are rich sources of essential nutrients such as carbohydrates, proteins, vitamins, minerals and dietary fibre (McDaniel and Jadeja, 2019). Also, they confer various health benefits ranging from reduced risk of chronic illnesses such as cardiovascular diseases and cancers to improving the overall consumer well-being (Linares-Morales *et al.*, 2018). Thus, governmental health agencies recommend daily inclusion of fresh produce in diets for healthier lifestyle. For example, the

U.S. Department of Agriculture encourages consumers in the U.S. to increase the consumption of fresh produce by over 100% (approximately 4.5 cups) for a two thousand calorie diet per day (USDA, 2015). Similarly, the UK's national food guide, the Eatwell Guide recommends that people eat at least 5 portions of a variety of fruits and vegetables every day in order to have a healthy lifestyle (PHE, 2016).

Fresh produce serve as suitable substrates for the growth of a wide array of pathogenic microorganisms such as bacteria, parasites and viruses (Erhirhie *et al.*, 2020). Consequently, fresh produce consumption has been linked to foodborne outbreaks of microbial infection which has increased over the years (Carstens *et al*., 2019). Some implicated fresh produce include lettuce, spinach, tomato, apple, carrot and grape (Yoon and Lee, 2018). Notable foodborne pathogens associated with fresh produce consist of bacteria (e.g. *Escherchia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*); parasites (e.g. *Cryptosporidium* spp. and *Cyclospora* spp.) and viruses (e.g. *Hepatitis* A and *Norovirus*) (Balali *et al.*, 2020). Symptoms of foodborne microbial infection range from abdominal pain, diarrhoea, fever, headaches, muscle aches and vomiting, to severe and life-threatening health problems such as autoimmune complications, enterotoxin poisoning, meningitis, septicaemia, haemorrhagic colitis, haemolytic uremic syndrome (HUS), and also miscarriage in pregnant individuals (Yeni *et al*., 2016).

Contamination by foodborne pathogens can occur at any point of the food value chain i.e. crop growth, harvesting, processing, storage or distribution (Iwu and Okoh, 2019). Various decontamination strategies have been used to improve the safety of fresh produce including chemical methods (e.g. chlorine, hydrogen peroxide and organic acids), physical methods (e.g. cold plasma, irradiation, non-thermal plasma and pulsed light) and the hurdle approach - a combination of different techniques. However, efficacy of decontamination is limited as none of these methods can ensure complete elimination of pathogens especially on a commercial scale (Bhilwadikar *et al*., 2019). Also, due to the problem of adhesion and internationalisation of these pathogens, control strategies to help significantly minimize the possibility of contamination and predisposition of fresh produce are still needed from farm to fork (Allende an Ölmez, 2015).

Several traditional subtyping techniques have been used for identification of foodborne pathogens and traceback investigations including, Polymerase Chain Reaction (PCR), Pulsed-Field Gel Electrophoresis (PFGE), Multilocus Variable-Number Tandem Repeat Analysis

(MLVA) and antigen-based assays. However, these methods have many drawbacks as they can be slow, cumbersome and lack specificity (Beeton-Kempen, 2019). Recently, rapid techniques like Whole Genome Sequencing (WGS) have been developed to precisely define foodborne outbreaks faster and effectively, though they can be relatively expensive. Moreover, there is need to adopt more rapid techniques such as immunoassays and nucleic acid- based methods for foodborne pathogen detection in order to establish patterns that can aid in the reduction and possible prevention of foodborne outbreaks of microbial infection (Makinde *et al*., 2020).

Despite the increase in fresh produce associated outbreaks, global food trends indicate an increase in consumer preference for fresh produce (Mason-D'Croz *et al.*, 2019). In fact, total global production of fresh fruit increased remarkably by 50.54% from 576.65 to 868.1 million metric tons between 2000 and 2018 (Statista, 2020). However, microbiologically safe supply of fresh produce on an industrial scale continues to be a major challenge in many parts of the world, particularly in the European Union (EU) and the US whilst representing a significant public health and economic issue for both regions (Callejón *et al*., 2015). Therefore, this study aims to review data on foodborne outbreaks associated with the consumption of fresh produce across Europe and North America over the last two decades; by highlighting the magnitude of the outbreaks, most prevalent pathogens and most implicated products while proffering solutions with a view to preventing future outbreaks.

MATERIALS AND METHODS

Data sources and literature search

The PRISMA and ORION statements were used in the search and the analysis process (Stone *et al*., 2007 and Liberati *et al.*, 2009). Literature searches were carried out using the advanced search engines of 2 journal databases (PubMed and Scopus) for the identification of peer-reviewed articles reporting outbreaks associated with fresh produce in the Europe and North America. Search terms include: "foodborne" AND "outbreaks" AND "fresh produce" and their variations. The search was conducted between March and April 2020.

Inclusion criteria

The screening process involved reviewing titles and abstracts of selected articles. Relevant articles were screened further by checking the full text while selected articles were checked for relevant references not obtained from direct searches. For the purpose of this study, a foodborne infectious outbreak is an illness that impacts two or more individuals as a result of

ingestion of a common food. Hence, single case studies were not included in this review. Foodborne outbreaks of microbial infection included those published in peer-reviewed journals up until the end of 2019. Forty-eight (48) articles met the inclusion criteria and were included in the systematic review. They all fulfilled the criteria for outbreaks linked to fresh produce consumption. All study design types were included while studies not meeting 5 or more of the following inclusion criteria were excluded:

1. Identification of pathogen involved in the outbreak
2. Number of people infected by the outbreak
3. Country where the epidemic occurred
4. Identification of implicated food
5. Outbreak cases were laboratory confirmed
6. Year of outbreak occurrence
7. Number of hospitalizations
8. Number of reported deaths

Fresh produce categories

Table 1 shows the different categories of fresh produce involved in foodborne outbreaks of microbial infection.

RESULTS

Selection process and search results

The article selection process is outlined in Figure 1. The literature search generated total hits of 725 of which 398 was for Scopus and 327 for PubMed. After initial review of all 725 studies, 604 articles were discarded after title and abstract review. 35 duplicates were discarded while 38 articles were further discarded after the data extraction process, as they did not meet the eligibility criteria set. The publications were related to (i) foodborne outbreaks of microbial infection (ii) laboratory investigation methods (iii) decontamination methods of fresh produce and (iv) risk assessment/management of fresh produce. From these articles, 48 research articles met the inclusion criteria and reported a total of 276 fresh produce-outbreaks.

Characteristics of included studies

A summary of the reported outbreaks of microbial infection associated with fresh produce from 1999 to 2019 in both regions is shown in Table 2. During the first two decades of the century, a total of 276 outbreaks of microbial infection associated with fresh produce were recorded with 44,524 individual cases, 3,152 hospitalizations and 198 deaths. The EU

recorded a total of 44 fresh produce-associated outbreaks with 22,236 cases, 1242 hospitalizations and 59 deaths while North America reported a total of 232 fresh produce-associated outbreaks with 22,288 cases, 1,910 hospitalizations and 139 deaths. The largest outbreak in Europe occurred in Germany in 2012 which was shown to be caused by *Norovirus* associated with frozen strawberries, affecting 10,950 people. However, North America recorded the largest outbreak which was caused by *Salmonella* saintpaul in 2008 when 1,442 people were infected through consumption of jalapeño peppers, resulting in 2 deaths.

A wide spectrum of foodborne pathogens was involved in outbreaks associated with fresh produce during the period of study (Figure 2). They included *Campylobacter*, *Cryptosporidium, Cyclospora*, *Escherichia coli*, *Hepatitis* A, *Listeria, Norovirus*, *Salmonella*, *Shigella* and *Yersinia*. *Cryptosporidium* was responsible for most of the outbreaks in Europe with 9/44 (20.5%) outbreaks whilst *Salmonella* was the main causative agent in North America with 121/232 (52.2%) occurrence. As shown in Table 1, different categories of fresh produce have been associated with the outbreaks. Figure 3 shows that vegetables are the most involved as food vehicles of outbreaks in Europe and North America with frequencies of 34.1% and 47.4% respectively. The yearly distribution of fresh produce outbreaks for the past two decades is shown in Figure 4**.** Considering the time frames within the period of study (1999-2005) and (2013-2019), there was an increase of 4 to 16 outbreaks and 69 to 99 outbreaks of fresh produce in Europe and North America respectively.

Outbreaks by Foodborne Pathogens

- **Outbreaks caused by bacteria**

Various bacteria were associated with fresh produce outbreaks from 1999 to 2019 including *Campylobacter*, *Escherichia coli, Listeria*, *Salmonella*, *Shigella* and *Yersinia* (Table 2). In Europe, a total of 25 outbreaks occurred with 6,553 cases, 140 hospitalizations and 57 deaths which were attributed to bacterial agents. *E. coli* and *Salmonella* both had the highest frequency of occurrence (18.2%) with the highest number of cases attributed to *E. coli*, a total of 4,628. In North America, the total number of bacteria outbreaks was 177 with 16,275 cases, 1,785 hospitalizations and 139 deaths. *Salmonella* was responsible for the highest bacterial outbreak of 13,651 cases and the most frequent outbreak (52.2%).

- **Outbreaks caused by viruses**

Viruses associated with fresh produce outbreaks of infection over the last twenty years included *Hepatitis* A and *Norovirus* (Table 2). In Europe, a total of 7 viral outbreaks from

fresh produce occurred with 13,923 cases, 1,102 hospitalizations and 2 deaths. *Hepatitis* A had the highest frequency of occurrence (11.4%) whilst *Norovirus* outbreaks had the highest number of cases being 12,062. In North America, a total of 17 viral outbreaks occurred with 793 cases, 69 hospitalizations and no deaths reported. *Norovirus* was responsible for both the highest viral outbreak and most frequently occurred viral outbreak with 474 individual cases and 5.6% occurrence respectively.

- **Outbreaks caused by parasites**

Parasites associated with fresh produce outbreaks from 1999 to 2019 included *Cryptosporidium* and *Cyclospora* (Table 2). In Europe, parasites were responsible for a total of 12 outbreaks with 1,760 cases and no hospitalization or death reported. *Cyclospora* was responsible for both the largest outbreak and the most frequently occurred outbreak caused by parasites with 1,695 cases and 20.5% occurrence respectively. North America recorded a total of 39 outbreaks due to parasites with 5,220 cases, 56 hospitalizations and no deaths reported. *Cyclospora* was identified as the outbreak causing the greatest number of cases (5,076) and was the outbreak most frequently occurring (16%).

Outbreaks by Food Vehicle

- **Fruits**

A total of 8 (18.2%) outbreaks in Europe associated with fruit consumption resulted in 12,964 cases, 1,102 hospitalizations and 2 deaths. Frozen strawberries were the responsible vehicles for the largest outbreak due to *Norovirus* with a total of 10,950 cases. In North America, 96 (42.4%) outbreaks were associated with fruit consumption which resulted in 9,444 cases, 937 hospitalizations and 112 deaths. Cucumber contaminated with *Salmonella* spp. was the vehicle responsible for the largest outbreak of 991 cases and 6 deaths.

- **Vegetables**

In Europe, 15 (34.1%) outbreaks were associated with the consumption of vegetables which resulted in 5,385 cases and 53 deaths. Vegetable sprouts were linked to the largest outbreak due to *Escherichia coli* O104:H4 with a total of 3,842 cases and 53 deaths. In North America, 110 (47.4%) outbreaks were associated with consumption of vegetables which resulted in 9,019 cases, 815 hospitalizations and 24 deaths. Mung bean sprout contaminated with *Salmonella* spp. was linked to the largest outbreak of 592 cases.

- **Salads**

A total of 13 (29.6%) outbreaks were linked to the consumption of salad in Europe with 2,194 cases, 66 hospitalizations and no deaths reported. Pre-cut mixed salad was the vehicle

of transmission for the largest outbreak resulting in 648 cases caused by *Cryptosporidium parvum*. However, a total of 7 (3%) outbreaks were linked to the consumption of salad in North America with 250 cases, 9 hospitalizations and 1 death. The largest outbreak resulted in 162 cases due to consumption of bagged salad mix contaminated with *Cyclospora cayetanensis*.

- **Juices**

Only 1 (2.3%) outbreak was linked to the consumption of juice in Europe. The outbreak was linked to raspberry juice due to contamination by *Cyclospora cayetanensis* which resulted to 13 infected cases. However, 2 (0.9%) outbreaks were reported in North America with the largest outbreak of 144 infected cases linked to the consumption of ozonated apple cider due to contamination by *Cryptosporidium parvum.*

- **Peas**

In Europe, 2 (4.6%) outbreaks were associated with consumption of peas which resulted in 35 cases. Snap peas and fresh sugar peas were the vehicles of contamination such that *Cyclospora cayetanensis* resulted in 18 cases and *Shigella sonnei* resulted in 17 cases. In North America, 3 (1.3%) outbreaks were associated with consumption of peas which resulted in 163 cases. The largest outbreak was linked to snow peas due to contamination by *Cyclospora cayetanensis* which resulted in 96 cases.

- **Mixed items**

These are outbreaks that had no clear distinction of the outbreak vehicle. Outbreaks due to mixed vehicles in Europe were 3 (6.8%) which resulted in 315 cases, 74 hospitalizations and 4 deaths. The largest outbreak was linked to the consumption of leeks and potatoes with a total of 250 cases, 74 hospitalizations and 1 death due to contamination by *Escherichia coli* O157. In North America, 4 (1.7%) outbreaks were recorded in relation to mixed vehicles with 743 cases, 57 hospitalizations and no deaths reported. The largest outbreak was caused by consumption of cilantro and salad mix as a result of *Cyclospora cayetanensis* contamination which led to 631 cases and 50 hospitalizations.

- **Other items**

Other items include fresh produce vehicles that do not fall under the above categories as shown in Table 2. In Europe, 2 (4.6%) outbreaks that resulted in 1,330 cases fall under this category. Interestingly, chilli product was the vehicle responsible for the largest outbreak of 1,112 cases caused by *Norovirus*. However, North America recorded a total of 10 (4.3%) outbreaks with 2,382 cases, 92 hospitalizations and 2 deaths. Jalapeño pepper was the

implicated vehicle for the largest outbreak due to contamination by *Salmonella* saintpaul which resulted in 1,442 cases and 2 deaths.

DISCUSSION

In the past 2 decades, fresh produce outbreaks of microbial infection have been on the increase especially in industrialized areas like Europe and North America. This increase can be attributed to increased consumption of fresh produce and recommendation for healthier lifestyles (Machado-Moreira *et al*., 2019). According to Wadamori *et al.* (2017), popular causes of fresh produce outbreaks of microbial infection include pathogenic bacteria like *Campylobacter spp*., *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* spp. and *Staphylococcus aureus*. Also, Chatziprodromidou (2018) reported that *Norovirus* and *Hepatitis* A are viral pathogens commonly associated with fresh produce consumption. Furthermore, Callejón *et al*. (2015) reported that *Norovirus* was responsible for produce related outbreaks (59% in the US and 53% in the EU) followed by *Salmonella* (18% in the US and 20% in European Union). This is similar to our findings that *Norovirus* and *Salmonella* have been associated with the highest number of infected cases from fresh produce in both regions.

Cyclospora and *Cryptosporidium* are two human parasites responsible for a notable link with fresh produce outbreaks. In this study, outbreaks linked to *Cryptosporidium* were commonly reported in Europe while *Cyclospora* outbreaks where mostly reported in North America. Likewise, Almeria *et al*. (2019) stated that *Cyclospora cayetanensis* outbreaks have been mostly reported in North America, probably due to advancement in detection methods and disease surveillance that have helped in tracking outbreaks.

This study revealed different categories of fresh produce responsible as vehicles in outbreaks of microbial infection such as fruits, juices, peas, salads and vegetables. However, leafy-green vegetables have been mostly implicated in these outbreaks. Similarly, Herman *et al*. (2015) reported that outbreaks associated with leafy green vegetables were greater than other types of food. This could be due to their high-water content, which makes them highly perishable and subject to colonization by enteric pathogens (Olaimat and Holley, 2012).

Consumers ensure intake of vegetables through processed fresh produce such as ready-to-eat (RTE) salads. However, analysis of RTE salads reveal poor microbiological quality which makes microbiological safety of fresh produce throughout the food value chain of more concern (Mogren *et al*., 2018). A major source of microbial contamination has been linked to inappropriate practices on the farm (Julien-Javaux *et al*., 2019). Pre-harvest sources of

contamination include contaminated seeds, irrigation water, soil, and pests while postharvest sources of contamination include machine contact surfaces, transport containers, insects and improper handling (Balali *et al*., 2020). A study conducted by Islam *et al*. (2004a) and Islam *et al*. (2004b) revealed that *E. coli* O157:H7 and *Salmonella typhimurium* can be transferred from contaminated soil and water to the surface of lettuce and parsley leaves. Even though washing fresh produce with potable water helps to remove microorganisms, the efficacy however, is limited (0.5–2.0-log reduction) which can be attributed to microbial attachment to surfaces or irregular surface structures of fresh produce (Banach *et al*., 2015).

In order to improve the safety of fresh produce, chlorine is mostly adopted by industries during washing due to its low cost and relatively good decontamination efficacy (Deng *et al*., 2019). Unfortunately, this practice presents several drawbacks: leaving disinfectant residues on fresh produce while producing toxic by-products (Tudela *et al*., 2019). Recently, researchers have focused on other alternatives for microbial decontamination of fresh produce such as the hurdle approach which seems to be promising due to the synergistic antimicrobial effect without significant detrimental effects on produce sensory quality (Bhilwadikar *et al*., 2019). Huang *et al.* (2018) also demonstrated a reduction in the incidence of cross- contamination between inoculated and uninoculated lettuce leaves with the use of ultrasound- assisted chlorine washing.

The challenge of fresh produce being a reservoir of foodborne pathogens is of greater concern due to the emergence of antimicrobial resistant (AMR) strains in fresh produce, thus making treatment of infections in patients even more challenging (Iwu and Okoh 2019). Prevention and control of foodborne infections requires the use of high- throughput molecular methods like WGS for public health surveillance and monitoring exercises (Makinde *et al.*, 2020). However, there is still need for development of more rapid and reliable detection methods with accelerated validation and worldwide acceptance which will aid timely outbreak investigations (Yeni *et al.*, 2014). Furthermore, implementation of good agricultural practices, proper risk prevention practices and alternative decontamination methods are recommended for prevention of fresh produce outbreaks microbial infection (Murray *et al*., 2017).

Limitations

This systematic review has focussed on the distribution of fresh produce outbreaks of microbial infection in Europe and North America. The selection of articles could be at risk of considerable publication bias due to the data sources, literature search and inclusion criteria used for the study, which has an implication on the results and conclusions. The present

review lacks details of some studies such as symptoms, laboratory confirmed cases and clinical investigations carried out due to difficulty in accessing the supported documents. These details are critical for the development of future prevention measures. The timescale of the present review was restricted to ensure comparability of laboratory methods, i.e. omission of studies before 1999. Furthermore, foodborne outbreaks of microbial infection that provide high-quality evidence of long-established causes and exposure routes may not have reached the peer-reviewed literature meaning that some of the outbreaks might be underrepresented in this systematic review.

CONCLUSIONS

This study has established the potential of fresh produce to harbour foodborne pathogens and cause outbreaks of microbial infection. In the past two decades, increase in consumption of fresh produce correlates with increase in fresh produce outbreaks of microbial infection in Europe and North America. Pathogens associated with these outbreaks include a range of microorganisms such as bacteria, viruses and parasites with *Cryptosporidium*, *Norovirus* and *Salmonella* playing important roles. Major categories of fresh produce involved in fresh produce outbreaks include fruits, vegetables, salads, juices and peas. However, vegetables were identified as the most implicated fresh produce category in both regions. Fresh produce outbreaks of microbial infection remain a major public health concern for the food value chain. Maintaining the microbial safety of fresh produce requires a systematic approach that encompasses implementation of good agricultural practices, proper risk prevention practices and alternative decontamination methods. Furthermore, more rapid methodologies are needed for timely traceback investigations in order to determine trends and epicentres of foodborne outbreaks relating to fresh produce which will help inform policies and initiatives important to public health and economy of the regions.

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TABLES

Table 1: Categories of fresh produce involved in foodborne outbreaks.

Category	Food vehicle
Fruits	Strawberries, mangoes, berries, berry mix, frozen berries, frozen strawberries, tomatoes, small tomatoes, semi dried tomatoes, dessert berries, raspberries, blackberries, apples, cantaloupe, stone fruit, caramel apple, honeydew, watermelon, musk, orange, citrus, grape, papaya, cucumber, avocado, melon and pre-cut melon.
Vegetables	Parsley, peeled carrots, sprouts, watercress, mung beans, alfalfa sprouts, baby spinach, basil, leafy greens, carrots, grated carrots, fresh vegetables, fresh spinach, Thai basil, cilantro, fresh basil, organic basil, lettuce, coleslaw, scallions, romaine lettuce and carrot mix, raw clover sprouts, cabbage, chopped celery, frozen vegetables, bean sprouts, mung sprouts, potato, raw alfalfa sprout and onion.
Salad	Salad, green salad, arugula salad, salad mixture, Frisée salad, pre-cut mixed

	salad, mixed salad, fruit salad, salad garnish, mixed salad leaves, rocket salad, salad vegetables, RTE salad mix, berry salad, bagged salad mix, chicken salad, coleslaw, chopped salad kits and packaged salad.
Peas	Sugar snap peas, raw peas, fresh sugar peas and snow peas.
Juices	Raspberry juice, ozonated apple cider and strawberry smoothie
Mixed items	Leeks and potatoes, lettuce and cucumber, basil in chicken pasta and tomato basil salad, mango and basil, cilantro and salad mix, RTE salad and cucumber,
Others	Chilli product, baby corn, pomegranate seeds, red chilli pepper, mushroom, raw produce, pepper and, pine nuts, coconut.

Table 2: Occurrence of foodborne pathogens linked to fresh produce outbreaks in Europe and North America.

Foodborne Pathogens		**Frequency of outbreaks**	
		Europe	**North America**
Bacteria	*Campylobacter*	-	1 (0.43%)
	Escherichia coli	8 (18.18%)	43 (18.53%)
	Listeria	-	12 (5.17%)
	Salmonella	8 (18.18%)	121 (52.16%)
	Shigella	4 (9.09%)	-

	Yersinia	5 (11.36%)	-
Viruses	*Hepatitis A*	5 (11.36 %)	4 (1.72%)
	Norovirus	2 (4.55%)	13 (5.60%)
Parasites	*Cryptosporidium*	9 (20.45%)	1 (0.43%)
	Cyclospora	3 (6.82 %)	37 (15.95%)
	TOTAL	**44**	**232**

LEGENDS TO FIGURES

Figure 1: Article selection process for existing studies on fresh produce outbreaks of microbial infection.

Figure 2: Distribution of foodborne pathogens linked to fresh produce outbreaks of microbial infection in Europe and North America.

Figure 3: Distribution of foodborne outbreaks of microbial infection among various categories of fresh produce in Europe and North America.

Figure 4: Foodborne outbreaks of microbial infection in Europe and North America from 1999 – 2019.

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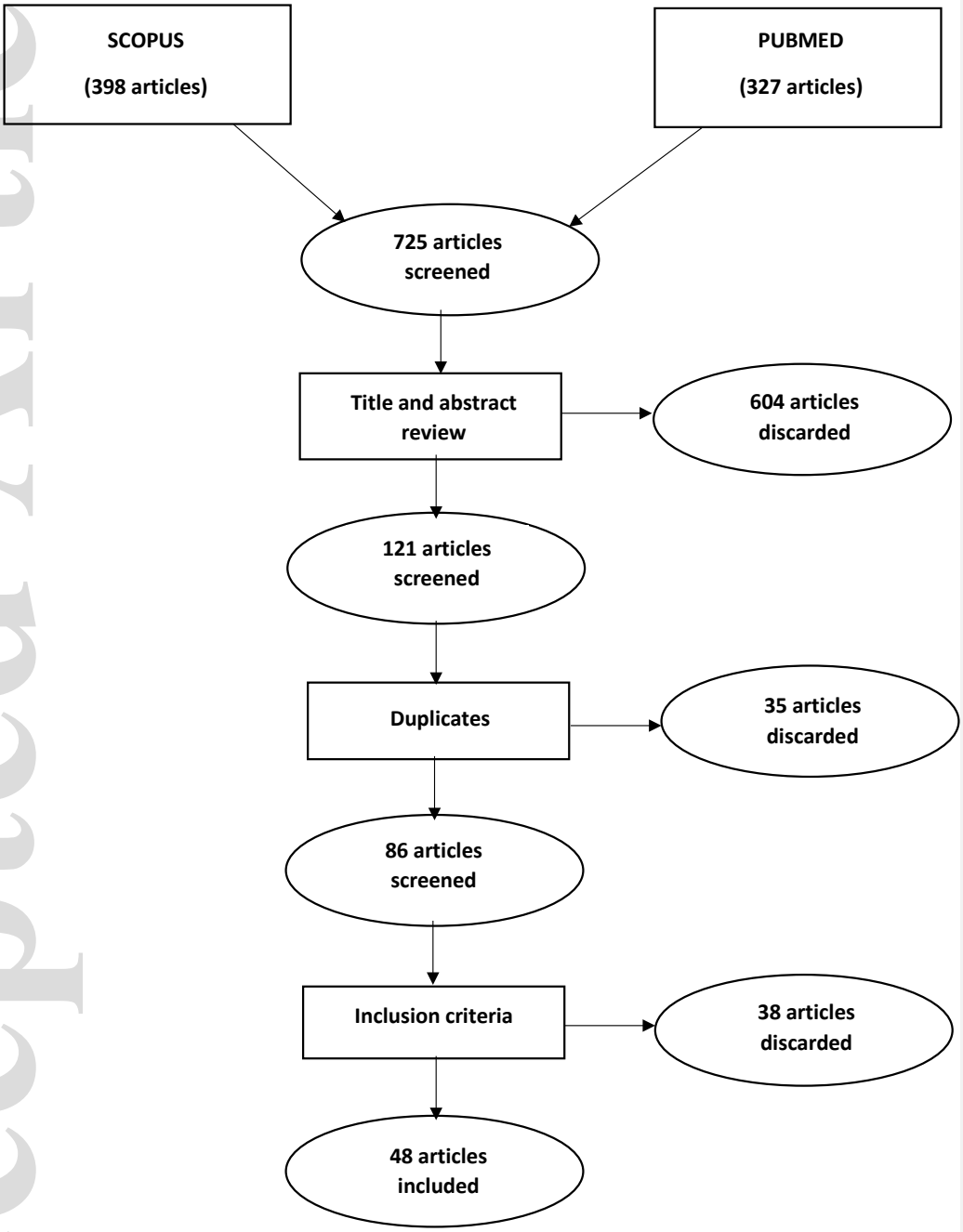
FIGURE 1



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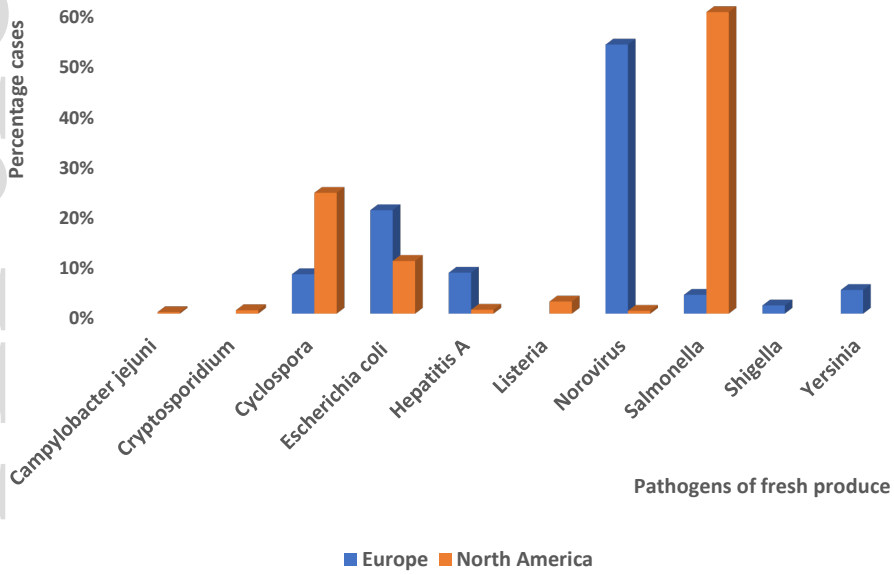

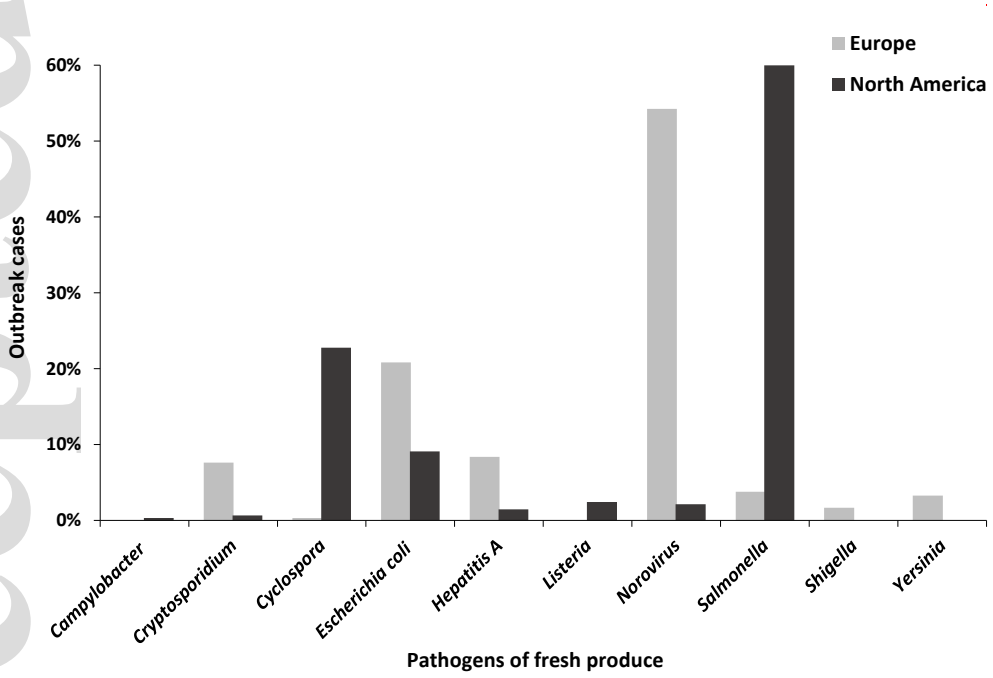

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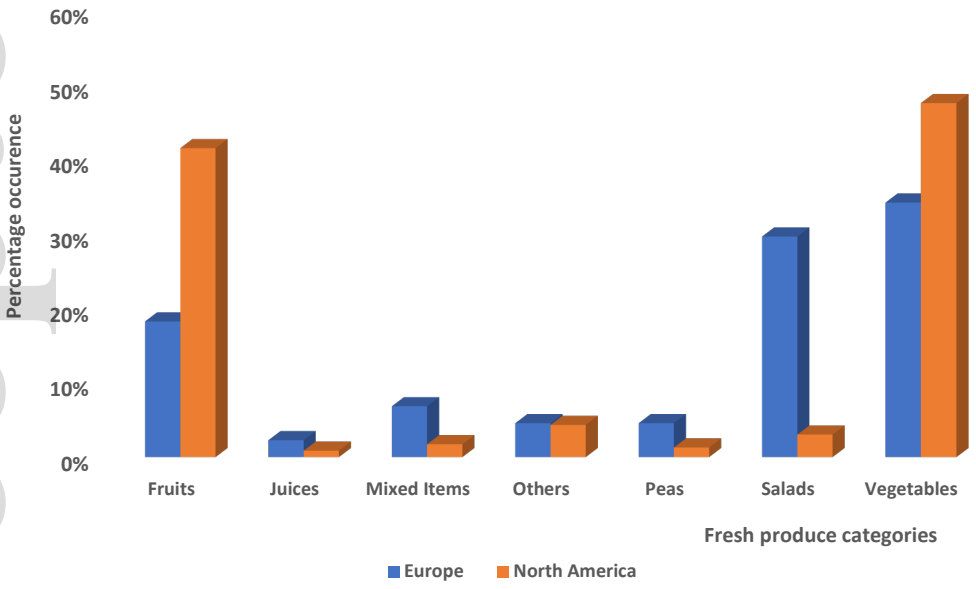

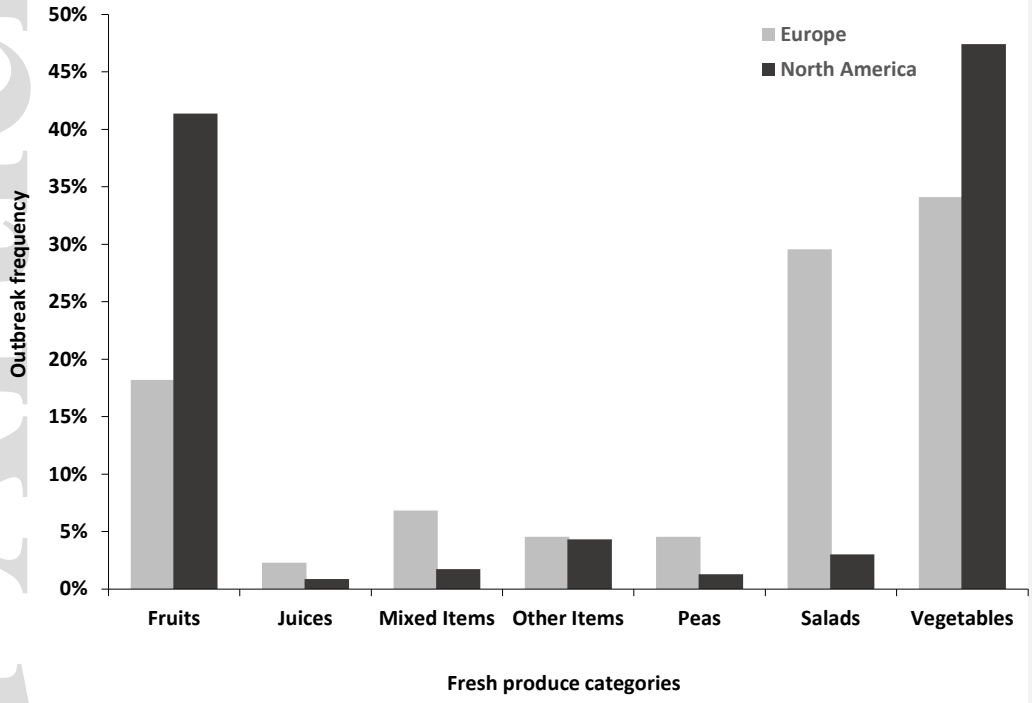

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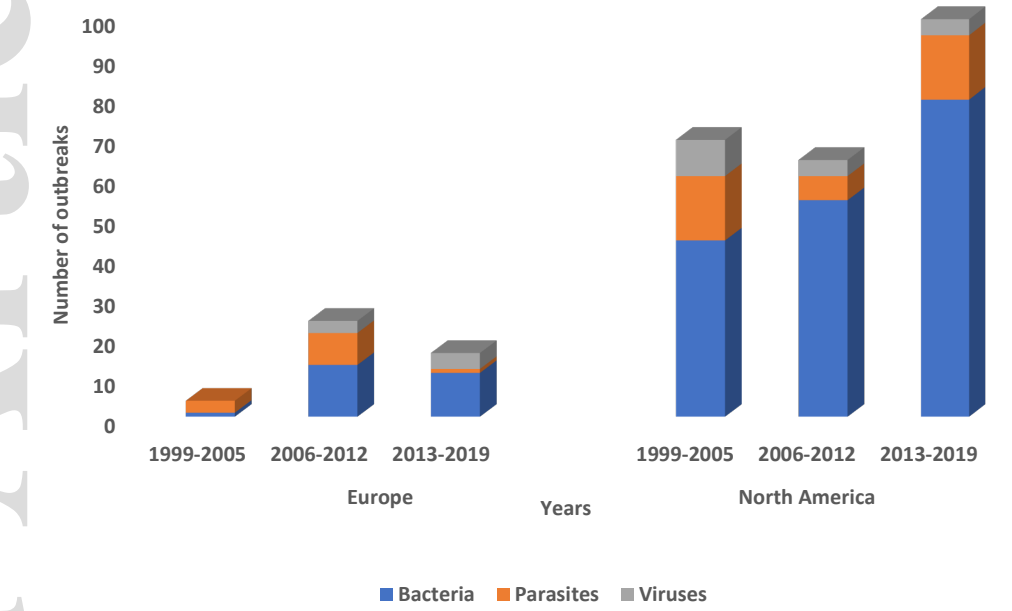

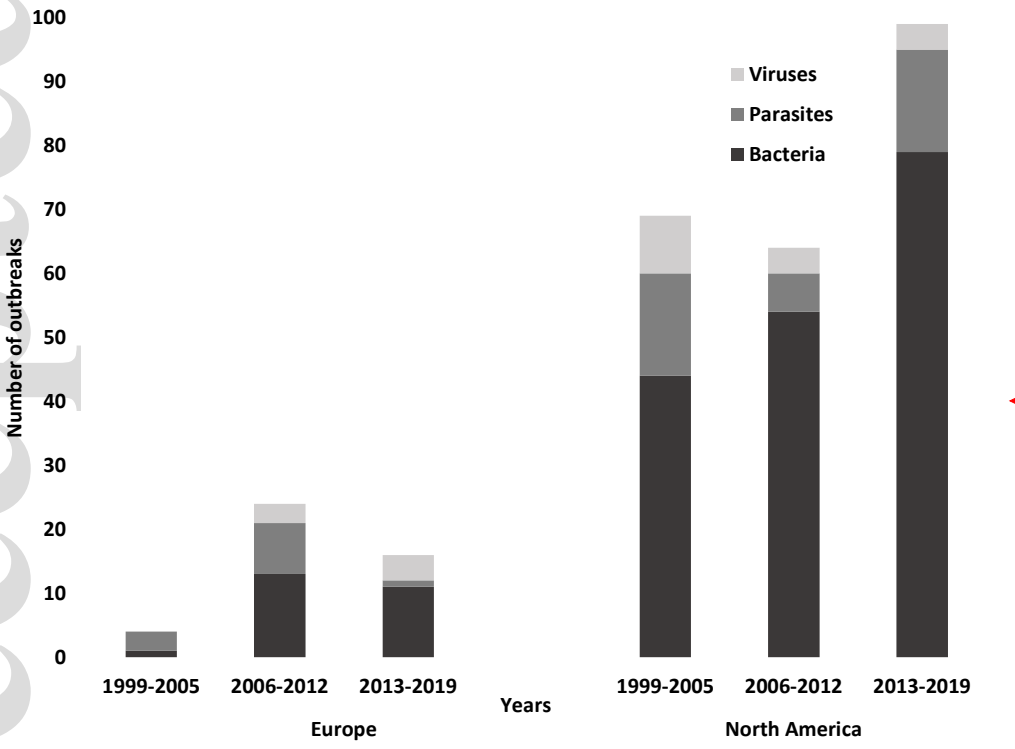

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